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12 Phylum Nematoda

Kálmán Molnár[1], Kurt Buchmann[2], Csaba Székely[1]
[1]Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, Hungary; [2]Department of Veterinary Microbiology, Royal Veterinary and Agricultural University, Stigbojlen 7, DK-1870 Frederiksberg C, Denmark

Introduction

Parasitic nematodes constitute one of the earliest known groups of helminths in fishes. They infect freshwater, marine and brackish-water fish species and sometimes cause substantial damage to the host. Although parasitic nematodes can infect almost all organs in a fish, the majority of the currently known species have been described from the intestine. Most nematodes infect fish as adults, but a large proportion of them occur as larval stages. These are usually parasites of piscivorous birds, mammals or reptiles, or less frequently of predatory fishes.

The majority of nematodes reach sexual maturity through a complicated developmental cycle involving an intermediate or possibly paratenic hosts. Species living in the temperate zone are usually characterized by seasonal occurrence, and annual life cycles are common. Because of the complicated, multi-host life cycle, the development of fish nematodes is successful in non-disrupted ecosystems. In fish taken from their natural surroundings, nematode infections are less likely to develop. For these reasons, nematodes cause less damage in cultured fishes than do other helminths. At the same time, certain nematodes can give rise to massive infections with high fish mortality in natural waters.

Although nematodes are important pathogens, their direct pathogenic effect on fishes is much less important than the role played by them as causative agents of zoonoses. Nematode infections in marine fishes cause a range of problems. Some of these are associated with pathogenicity of the parasite to the fish host, while others are health hazards connected to human ingestion of live nematodes in fresh or undercooked fish. Furthermore, consumer attitudes to the presence of nematodes in foods also have a great impact on the market value of fish products and may stress the need for strict monitoring and diagnosis of nematode infections in marine fish stocks.

The prevalence of parasitic nematodes on the different continents is not equally well known. The majority of species have been described from Europe. Detailed data on nematodes of freshwater fishes (Avdeev *et al.*, 1987; Moravec, 1994) and those in North American fishes (Hoffman, 1999) are readily available. Recently major progress has been made in the study of fish-parasitic nematodes in the Neotropical region (Moravec, 1998). Of the nematodes parasitizing marine fishes, anisakid species causing human infections are relatively well documented (Hauck and May, 1977; Smith and Wootten, 1978; Berland, 1981; Sindermann, 1990; Koie *et al.*, 1995). Relevant data on the

occurrence and pathological role of fish-parasitic nematodes are found in the first edition of this publication (Dick and Choudhury, 1995). Since the publication of the work, relatively little new information has been added to our existing knowledge of fish-parasitic nematodes. The new data concern primarily the development, pathological effect and human health implications of a few selected groups (*Anguillicola*, *Philometra*, *Skrjabillanus*, *Anisakis*).

Economic Importance

In some cases, parasitic nematodes can produce very spectacular and massive infections in fishes. The masses of *Philometra ovata* (Fig. 12.1) in the abdominal cavity of gudgeon or the white-coloured *Hysterothylacium bidentatum* (Fig. 12.2) in the stomach of sterlet (*Acipenser ruthenus*) definitely indicate that nematodes are important pathogens. In spite of this, Williams (1967) rightly stated that among the helminth parasites of fishes the pathological effect of nematodes had been studied the least. The situation has not changed. Although the number of known species has gradually increased, there are still few new data on the pathological effects of nematodes. In freshwater fish species, the new data mostly concern *Anguillicola crassus* (Fig. 12.3) infection in eel (*Anguilla anguilla*). As regards marine fish parasites, the anisakid species (*Anisakis*, *Contracaecum*, *Hysterothylacium*, *Pseudoterranova*) have come into prominence because of infections in humans.

Fish nematodes might harm their host in a variety of ways. They can cause mechanical injuries, atrophy of tissues, occlusion of the alimentary canal, blood vessels and other ducts and toxication from their metabolic products, and they can deprive the host of food, enzymes and vitamins.

Fig. 12.2. *Hysterothylacium bidentatum* specimens in the stomach of the sterlet (*Acipenser ruthenus*) (× 1.2). Photo by György Csaba.

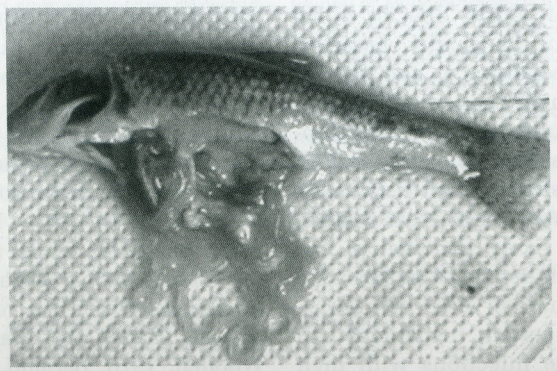

Fig. 12.1. *Philometra ovata* filling the abdominal cavity of a gudgeon (*Gobio gobio*) (× 0.6).

Fig. 12.3. Heavy and moderate infections with *Anguillicola crassus* in opened swim bladders of European eels (× 0.6).

Host Range

Nematode infection occurs in practically all fish species and in all locations. The prevalence, intensity and economic importance of nematode infections, however, vary by region and by fish species.

The host range of these parasites is primarily influenced by the host specificity of the nematode species for the final host and for the intermediate host. Species having a narrow host range are usually in a fish host that is prevalent in a given habitat, while species with a broad host range are in fish species all over the world. These nematodes can often cause mild disease in all members of a fish genus and, after colonizing a new host species, they can give rise to much more severe disease. Such a species is *A. crassus*, a nematode of Japanese eel (*Anguilla japonica*), which causes a mild disease in its original hosts. However, this nematode is much more pathogenic in European and American eel species (*Anguilla rostrata*).

The typical examples of highly specific nematodes are the members of different skrjabillanid genera (*Skrjabillanus tincae*, *Skrjabillanus cyprini*, *Molnaria intestinalis*, *Sinoichthyonema amuri*), which in most cases infect a single fish species.

Nematodes with a broad host range are represented by *Capillaria* species, which can colonize numerous fish species of different taxonomic positions. Fish parasites characterized by a global range include the larval stages of nematodes of seabirds and marine mammals in their adult stage (*Anisakis*, *Pseudoterranova*). The distribution of the majority of freshwater nematode species is mostly restricted to a single continent or specific zoogeographical zones thereof.

Systematic and Taxonomic Position

The phylum Nematoda consists of about 40,000 species of free-living and parasitic nematodes (Anderson, 2000). Nematodes occur also in fish, though the number of species infecting fishes is relatively few if they are compared with the terrestrial hosts, which are highly diverse and rich in taxa (Anderson, 1996). Phylum Nematoda is divided both by cladistic and molecular methods into two major classes, Adenophorea and Secernentea. Adenophorea consist mostly of free-living marine and freshwater species, as well as terrestrial soil nematodes with only a small number of parasitic organisms. These include three families: Dioctophymatidae, Capillariidae and Cystiopsidae. Secernentea also have free-living taxa, but the vast majority of this class are parasitic organisms.

Classification of fish nematodes in this chapter is based on Anderson's (2000) system, but some modifications have been made in lower taxons, as suggested by Moravec (1994, 1998).

Parasite Morphology and Life Cycle

Morphology

Nematodes in marine and freshwater fishes are highly variable in size, from microscopic to 10–20 cm in length, as with *H. bidentatum*, *Philometra obturans* and *Philometroides cyprini* (Fig. 12.4), and there are specimens as small as *Lucionema balatonense*. Adult nematodes are sexually dimorphic and they have five stages (L1, L2, L3, L4 and adult stage) with four moults. Most species have a fusiform shape, being widest in the middle and tapering at the anterior and posterior ends. Capillariids and skrjabillanids are typically fusiform with a uniform diameter and an extremely thin, elongated body (Fig. 12.5). Female *Cystoopsis acipenseris* have filiform anterior and globular posterior body regions. Nematodes have a cuticle and are without cilia. They do not have protonephridia, respiratory organs or blood systems.

Female nematodes are usually larger than males. While the size of female ascaridoids, camallanoids and capillariids are only rarely double the size of the males, philometrid females can be 100 times the length and up to 100,000 times the body weight of males. Most nematodes have a yellow or whitish colour but those that live in the blood vessels (*P. obturans*, *Philometroides sanguinea*) or feed on blood (*A. crassus*) may be a red or dark brown colour. Some intestinal nematodes may have a transparent body but with a distinct yellow buccal capsule (*Camallanus*).

The cuticle of nematodes is elastic, and it is thick in gut-dwelling species (*Hysterothylacium*, *Eustrongylides*) and relatively delicate in histozoic specimens (*Philometra rischta*, *Daniconema anguillae*). The surface of the cuticle may be smooth but it can bear longitudinal striations or transverse rows. The cuticle of some nematodes is covered entirely or partly with spines (*Spinitectus*, *Gnathosoma*), and distinct bosses might also cover the cuticular surface (*Philometroides nodulosa*, *P. cyprini*). The cuticle may also have papillae with a tactile function and which serve as chemoreceptors. All papillae are connected by nerve endings.

Fig. 12.5. Female specimen of *Capillaria pterophylli* in the gut of a discus fish (*Symphysodon discus*). (× 66) Photo by Ferenc Baska.

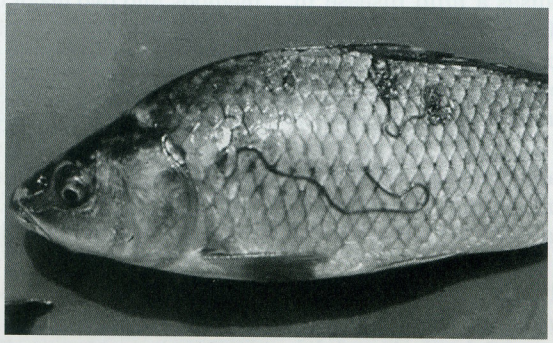

Fig. 12.4. *Philometroides cyprini* female freed from the scale pocket of common carp (*Cyprinus carpio*) (× 0.3).

Most papillae are located at the cephalic and caudal region. According to their location and function, they are called labial, cephalic, cervical and genital papillae.

The structure of the mouth shows great variations. It may be a simple slit-like opening at the anterior end surrounded by distinct or indistinct papillae (*Capillaria*, *Philometra*), but it can form large labia or cuticular outgrowths (Fig. 12.6), called interlabia (*Hysterothylacium*, *Anisakis*, *Raphidascaris*). The mouth leads into the buccal capsule (Figs 12.7 and 12.8), which can be sclerotized and furnished with large denticles, ridges, plates or tridents (*Camallanus*, *Cucullanus*, *Skrjabillanus*, *Anguillicola*). The buccal cavity is followed by the oesophagus. In some species a pharynx is located between the buccal capsule and the oesophagus. The structure of the oesophagus varies; in some groups (ascaridoids, camallanoids, etc.) it is entirely muscular, while in others (e.g. capillariids, philometrids) it has a muscular and a glandular part. The oesophagus joins the intestine directly or through a subglobular or elongated ventriculus. This ventriculus forms an elongated, posteriorly directed tube in ascaridids (*Raphidascaris*). Similar but anteriorly extending elongation of the intestine (intestinal caecum) exists in other ascaridids (*Pseudoterranova*, *Porrocaecum*) and in several genera of this group (*Contracaecum*, *Hysterothylacium*) both ventricular and intestinal appendices exist. The intestine is usually a straight tube finishing in the rectum. The rectum opens on the ventral side near the posterior end. In the males the rectum and the ejaculatory duct form a joint cloaca.

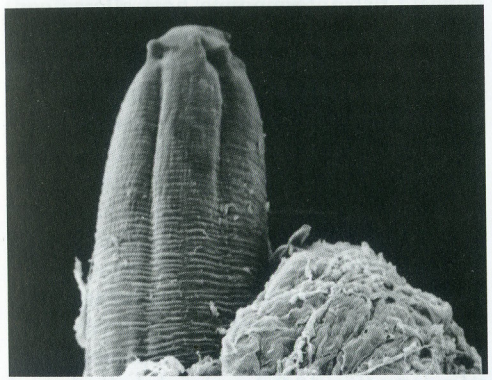

Fig. 12.6. Anterior end of *Anisakis simplex* with interlabia around mouth opening. Scanning electron microscope (SEM) (× 115). Photo by José Bresciani

Fig. 12.7. Buccal capsule of *Camallanus truncatus* from the gut of pike perch (*Sander lucioperca*) (× 115).

An oesophageal nerve ring represents the central nervous system, and special structures (papillae and dierids), located mostly in the anterior and posterior ends of body, serve a sensory function.

In some fish nematodes, there are no great differences in the general appearance of the two sexes and without microscopic examination males and females, especially juvenile forms, are hard to distinguish (*Anguillicola*, *Raphidascaris*). Other fish nematodes have striking differences between male and female, both in size and the structure of the tail (*Philometra*, *Skrjabillanus*, *Capillospirura*).

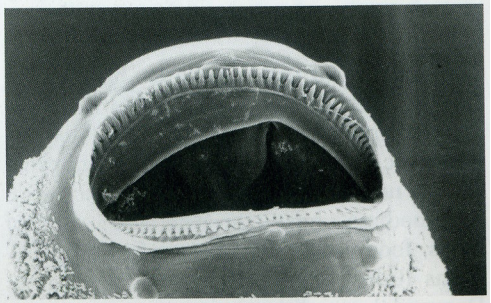

Fig. 12.8. The anterior part of *Cucullanus heterochrous* showing mouth opening. SEM (× 340). Photo by José Bresciani

The male reproductive organ usually consists of testis, vas deferens, seminal vesicle and ductus ejaculatorius. The ejaculary duct, opening into the cloaca, has some accessory copulatory organs. The most common accessory organs are the sclerotized spicules. Most fish nematodes have two spicules. They can be equal or different in size. The number of spicules in Capillariidae is one. No spicules exist in Anguillicolidae. The spicules are often supported by another sclerotized organ, the gubernaculum, which directs the movement of the spicules. In some skrjabillanids, a sclerotized copulatory plate substitutes for the spicules. A typical bursa copulatrix with membranous lateral alae forms the copulatory apparatus of skrjabillanids and a similar structure is found in male *Capillospirura*.

The female reproductive organs are composed of ovaries, oviducts, uteri, vagina and vulva. In most cases there are two sets of tubular ovaries locating anteriorly and posteriorly from the vulva opening. Ovaries continue to oviducts and uteri, from which eggs or larvae pass into a muscular common vagina. The vulva may be located in different parts of the fish body, characterizing families and genera of the nematodes. In *Capillaria*, *Skrjabillanus* and *Raphidascaris* spp., the vulva is found in the first part of the body length, but in *Camallanus* and *Rhabdochona* spp. it is situated postequatorially. The *Capillospirura* vulva is located approximately at mid-length. In adult *Philometra* specimens, the vulva and vagina are absent.

Development

The majority of fish nematodes have rather complicated development. Most are heteroxenous and have final, intermediate and paratenic (transport) hosts. In the life cycle of fish nematodes, there is always a single intermediate host. In the majority of cases, food animals such as aquatic crustaceans (Copepoda, Amphipoda, Mysidacea, Decapoda), annelids, coelenterates, molluscs and small fishes are intermediate hosts. Also parasitic organisms infecting fish, such as cyclostomes and crustaceans of the class Branchiura (fish lice), can transmit developmental stages to fish. Both fish and invertebrates may serve as transport (paratenic) hosts for some nematodes. A transport host is an organism in which no development necessary for the progress of the life cycle takes place. A high number of larvae can accumulate in such hosts. Although direct development without an intermediate host may be a possibility for some groups (e.g. for Capillariidae), this has not been unequivocally proven experimentally. Recently it was shown by Køie (2001c) that the marine *Capillaria gracilis*, which invades the rectum of Atlantic cod, needs intermediate hosts. Thus, chironomids and oligochaetes ingest the larvated eggs, where hatching and some growth of the larvae occurs. Further, the obligate fish intermediate host allows considerable growth of the worm before infection of the final host. In the case of direct development, host finding is often promoted by paratenic hosts, e.g. by oligochaetes carrying the larvated eggs (Bell and Beverley-Burton, 1980; Lomakin and Trofimenko, 1982; Moravec, 1983). Percutaneous transmission by migrating larvae, which is common in terrestrial animals, does not exist in fishes.

Some worms lay unsegmented eggs, and these worms are oviparous. Ovoviviparous worms lay eggs containing the first- or second-stage larva (Fig. 12.9a), and viviparous worms discharge free first-stage larva (Fig. 12.9b).

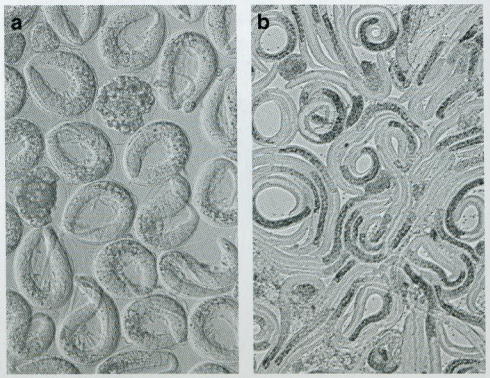

Fig. 12.9. (a) Ovoviviparous eggs of *Anguillicola crassus*. Second-stage larvae inside the extremely thin egg shells, (b) Viviparous larvae of *Philometra ovata* released from a ruptured female (× 85).

Thus the egg development, embryonation of the egg and/or subsequent survival of the larva are highly dependent on the environmental conditions. Hatching of eggs can occur in the environment after release with host faeces or after ingestion by intermediate hosts (oviparity), just after oviposition (ovoviviparity) or in the female worm (viviparity).

Final hosts of fish nematodes are both homeothermic and poikilothermic animals. In the case of some nematodes with a worldwide distribution, marine mammals and fish-eating birds serve as final hosts. Whales are the final hosts of the ascaridoid nematode *Anisakis simplex*, while for another marine ascaridoid, the seal worm *Pseudoterranova decipiens*, seals play the role of the final hosts. *Contracaecum* and *Porrocaecum* spp. have a wide host range and both birds and mammals can be final hosts.

The majority of the known fish nematodes develop to the adult stage in fish. Marine nematodes of this type include *Hysterothylacium aduncum*, *Cucullanus cirratus*, *Cucullanus heterochrous* and *Dichelyne* (*Cucullanellus*) *minutus*, while the genera *Capillaria*, *Camallanus*, *Rhabdochona*, *Philometra*, *Philometroides*, *Anguillicola* and *Raphidascaris* are in freshwater fishes and are regarded as the best studied and economically most important nematodes.

The most common route for infection of the intermediate host is when the eggs or free larvae are consumed by these animals. The first-stage larvae attract the attention of their intermediate hosts by their movement before being eaten by the intermediate host. Nematode larvae of the *Philometra*, *Anguillicola* or *Camallanus* genera penetrate into the haemocoel of the copepod intermediate host (Fig. 12.10) within a very short time and, depending on the water temperature, third-stage infective larvae are formed (Molnár, 1966b; Moravec, 1971; Hirose *et al.*, 1976). Non-motile eggs of *Rhabdochona*, *Cystidicoloides* or *Cystidicola* species are ingested by their mayfly or gammarid intermediate hosts. A special way of development in the intermediate host is when the vector is also a parasite. To date, only the branchiurid *Argulus* spp. (Fig. 12.11) and cyclostomes are known to transmit infection from one fish to another as intermediate hosts.

According to Moravec (1994), there is always a single intermediate host in fish nematodes, and host finding of nematodes is promoted by one or more paratenic hosts. The developmental cycle of capillariid nematodes seems to be monoxenous. Eggs, however, are often taken up by oligochaete paratenic hosts, in which the larvated eggs preserve their viability for a long time and serve as sources of infection. In the development of

Fig. 12.10. Third-stage larvae of *Anguillicola crassus* (arrows) in the haemocoel of the intermediate host cyclops (× 80).

Fig. 12.11. Third-stage larva of a *Skrjabillanus* sp. (arrow) entering the stylet of the intermediate host carp lice (*Argulus foliaceus*) (× 40).

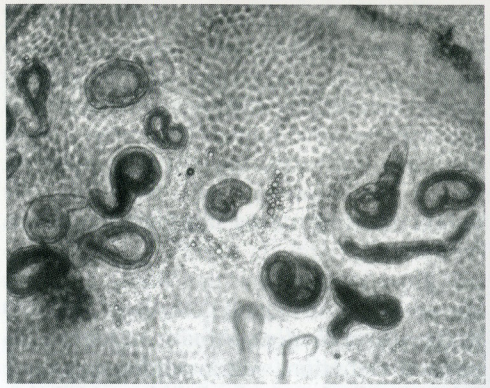

Fig. 12.12. Encapsulated dead and live third-stage larvae of *Anguillicola crassus* in the gut wall of the paratenic host ruffe (*Gymnocephalus cernuus*) (× 25).

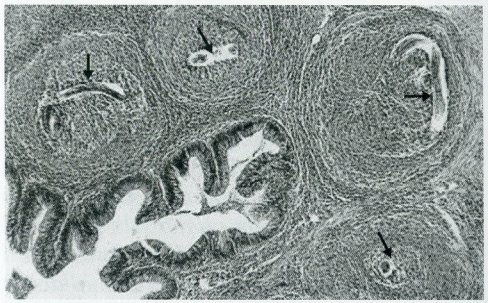

Fig. 12.13. Histological section of the gut of a fingerling of the paratenic host sheatfish (*Silurus glanis*). Third-stage larvae (arrows) of *A. crassus* encapsulated by epithelioid cells and connective tissue. Haematoxylin and eosin (H & E) (× 80).

the ascaridoid *Raphidascaris acus* oligochaetes, snails and crustaceans may serve as paratenic hosts before non-developing larvae get into the obligatory small fishes in which they reach the infective third stage. In this species the final host can also serve as intermediate host. For *A. simplex* the euphasiaceans, for *Contracaecum osculatum* and *P. decipiens* copepods and for *H. aduncum* copepods, amphipods, isopods and mysids can be the intermediate hosts (Smith and Wootten, 1978; Køie, 1993, 2001b; Køie *et al.*, 1995). It is also small fish which act as the true intermediate host for *C. cirratus* (Køie, 2000a), while polychaetes are intermediate hosts for *Cucullanus heterochrous* and *Dichelyne minutus* (Køie, 2000b, 2001a).

In most cases, paratenic hosts (crustaceans, snails, oligochaetes, small fishes and tadpoles) are food organisms of the final host. The role of paratenic hosts in the development of various nematode families is different. In the family Philometridae, the larvae of *P. obturans* from the copepod intermediate hosts are transmitted to the predacious pike through the prey fishes. The role of paratenic hosts is more important with the anisakids. Hosts (in the majority large predacious fishes, marine mammals and fish-eating birds) get infected with these parasites by several paratenic hosts. When a paratenic host is consumed, the infective third-stage larvae can survive to infect the new organism until they get to the final host. The process was studied in detail in *A. crassus* infection in European eel. The paratenic host fishes of this nematode may have massive infections (Figs 12.12 and 12.13) (Thomas and Ollevier, 1992; Székely, 1994, 1995).

Homeothermic animals as final hosts

A. simplex is an ascaridoid nematode with a worldwide distribution. The final hosts are whales, intermediate hosts are euphausiaceans and transport hosts can be copepods, fishes and cephalopods (Smith and Wootten, 1978; Køie, 2001b). In the final host, the nematodes reside in the stomach, while they are in the haemocoel in the intermediate crustacean host. In the fish transport hosts, the worms are in organs such as gonads, liver, spleen and the musculature. (Smith and Wootten, 1978). Adult worms copulate in the final host. Eggs are delivered to the sea with host faeces. The eggs embryonate and two moults occur in the egg before hatching. The larva is third-stage (Køie *et al.*, 1995) and, before getting into the obligatory euphausiacean intermediate host, the free-living, ensheathed larvae are ingested by transport hosts such as copepods. The intermediate host can also be ingested by various types of transport hosts (fishes, cephalopods) (see Køie, 2001b).

Another marine ascaridoid with a worldwide distribution is the seal worm *P. decipiens*. Final hosts are seals, intermediate hosts are copepods and benthic crustaceans. Fish may act as paratenic hosts. The site in fish is the musculature. Adult worms live in the stomach of the seal. Eggs pass from the intestine to the marine environment, where embryonation and two moults occur inside the egg. The third-stage larva emerges on hatching (Køie *et al.*, 1995). Copepods ingest the larvae. These can be eaten by benthic crustaceans. Invertebrates are eaten by fish, in which the muscle becomes infected. Seals ingest the fish and final moults to the adult stage take place in the seal.

C. osculatum also has a worldwide distribution and uses warm-blooded animals (seals) as final hosts. Intermediate hosts are crustaceans and fish carry the third-stage larva (Køie and Fagerholm, 1995). A number of fish species, such as cod, salmon, sculpin, herring and others, can be infected (Fagerholm, 1982; Hemmingsen *et al.*, 1993). In gadoids the liver is the preferred site (Fagerholm, 1988). Adult female worms deliver eggs to the environment. Two moults occur inside the egg and the third-stage larva emerges from the egg. Before infecting marine crustaceans, copepods can act as transport hosts. Fish acquire infection by predation on crustaceans. In gadoids the liver carries the majority of larvae. Final development takes place after ingestion of transport hosts by warm-blooded animals.

Poikilothermic animals as final hosts

One widely distributed nematode in fishes is *H. aduncum*. Eel pout, *Zoarches viviparous*, is one of the best final hosts. However, the worm may be found in numerous other species, such as burbot, cod, flounder, four-horned sculpin and whitefish (Fagerholm, 1982). The digestive tract (stomach and anterior intestine) is the site of infection for the adult worm. Eggs from the adult female are passed with host faeces to the sea water, where embryonation takes place, with two moults in the egg. The third-stage larva develops in copepods, amphipods, isopods and mysids (Køie, 1993). Other organisms, such as ctenophores, chaetognaths, polychaetes and ophiurids, which obtain infection by ingesting infected crustaceans, can serve as transport hosts. Often prey fishes serve as transport hosts as well. Larvae may be found in the body cavity and encysted on various organs in the fish (Fagerholm, 1982). Larvae longer than 3 mm moult into the fourth stage within the intestinal lumen of the host.

The cucullanids have another variation: Atlantic cod, *Gadus morhua*, and other gadoids are the final hosts of *C. cirratus* in the North Atlantic and adjacent waters. The preferred microhabitats are the pyloric caeca and the intestine. Eggs pass with faecal matter to the sea water, where embryonation occurs. The third-stage larvae hatch from the eggs. Intermediate hosts are small fishes, such as sand gobies, which become infected by eating the transport host, copepods. When the final host, the cod, ingests the infected intermediate fish hosts, the third-stage larva invades the stomach mucosa, where moulting to the fourth stage occurs. Subsequently, this larva passes to the pyloric caeca and the anterior intestine, where the final moult takes place and the worm develops into the adult stage (Køie, 2000a).

A similar development is seen in *C. heterochrous*, a common worm of flatfishes in the Baltic, Atlantic and North Sea. Final hosts include flounder, dab, plaice, sole, halibut and long rough dab (Berland 1970). The site is the intestine, primarily the

posterior half of the intestine of the flounder (Buchmann, 1991). Eggs pass with host faeces to the sea water, where embryonation occurs. Probably the third-stage larva emerges from the egg following ingestion by a polychaete, which serves as intermediate host (Køie, 2000b). Larvae from polychaetes are infective to fish, where they moult to the fourth stage in the submucosa. After the final moult, the larva develops into the adult stage in the intestine (Køie, 2000b).

The related nematode *D.* (*Cucullanellus*) *minutus* is in flounder, plaice and goby in the Baltic Sea, North Sea, Mediterranean Sea and Black Sea (Køie, 2001a). It is in the anterior part of the intestine (Buchmann, 1991). Eggs from the female worm pass with faeces to the sea bottom, where embryonation takes place. Probably two moults occur in the egg, and the larva (440 µm long) that hatches from the egg is considered a third-stage larva (Køie, 2001a). The polychaete *Nereis diversicolor* is the obligate intermediate host. Following growth, the larva is infective to fish such as flounders. The fourth moult occurs in the gut wall of the final host and the adult worms reside in the anterior intestine.

Host–Parasite Relationship

Immune reactions

Nematodes elicit specific antibody production in the host. Migration of the larval stages in host cavities and host tissue may expose both structural and metabolic antigens to the host immune system. It has been demonstrated by immunoblotting that the European eel produces specific antibodies against a number of antigens of the swim bladder nematode *A. crassus* (Buchmann *et al.*, 1991; Höglund and Pilström, 1994; Békési *et al.*, 1997; Nielsen and Buchmann, 1997; Knopf *et al.*, 2000). Third-stage larvae of *A. simplex* in the fish host provoke the production of specific antibodies in naturally infected saithe (Priebe *et al.*, 1991). In addition, a range of Antarctic teleosts has been shown to possess reactive antibodies against molecules from *C. osculatum* (Coscia and Oreste, 1998). Similar positive plasma and bile antibody reactivities in fish against *P. decipiens* larvae were recorded by Coscia and Oreste (2000). Two main types of antigens are generally recognized in nematodes. These are the soluble excretory and secretory (E/S) antigens and the somatic antigens associated with surfaces on the outer or inner part of the worm. These may be partly protective. However, it is generally agreed that cellular reactions play a major role in protection of the host against nematode infections. Both humoral and cellular host reactions have been detected in various hosts against invading nematodes and these immune factors may function in host elimination and killing of infective stages. However, the roundworms seem to have some evasion mechanisms. Thus, the cuticle of nematodes consists in most cases of a protective proteinaceous layer, which protects the inner vital organs of the worm against aggressive immune effectors. Even encapsulated *Anisakis* and *Anguillicola* worms recovered from infected fish are fully capable of moving vividly upon removal of the host encapsulation (Santamarina *et al.*, 1994; Székely, 1994; Larsen *et al.*, 2002). These mechanisms have not been adequately investigated.

Pathogenicity

Pathological effect

The pathological effect of nematode infection in fish is little studied, and most information is based on field observations. There are only a few reported cases of mortality due to nematode infections. Most authors (Bauer *et al.*, 1977; Moravec, 1994; Dick and Choudhury, 1995) agree that fish nematodes damage the hosts by depriving the fish of digested food; by feeding on host tissues, sera or blood; and by direct mechanical damage through fixing to host tissues and developing or migrating in them (Fig. 12.14). Nematodes generally possess a range of enzymes, such as proteases, which may have tissue-degrading functions (Newton and Munn, 1999). Large-sized parasites compress organs (Platzer and Adams, 1967),

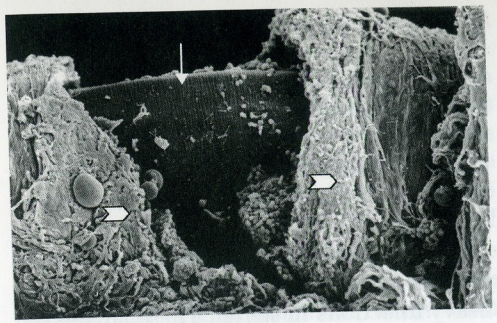

Fig. 12.14. *Anisakis simplex* (arrow) penetrating pyloric caecum of rainbow trout and causing mechanical damage of the wall (arrowheads) (experimental infection) (SEM × 50). Photo by José Bresciani.

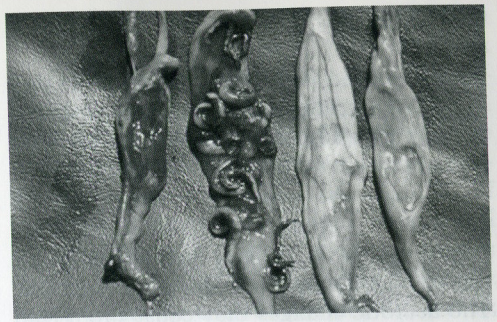

Fig. 12.15. Damaged swim bladders of the European eels that died during the eel mortality in 1991 in Lake Balaton. One of the swim bladders contains numerous *A. crassus* specimens, while others have thickened fibrous walls as a consequence of past infection (× 0.5).

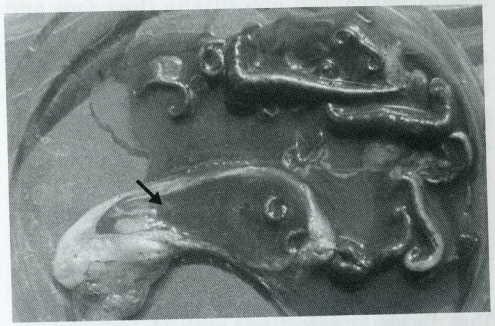

Fig. 12.16. With heavy infections with *A. crassus*, the worms die and decay in the lumen of the thickened swim bladder (arrow) (× 0.7).

deform the shape of the body (Molnár, 1966a), increase or reduce the size of organs (Paperna, 1974) and cause haemorrhages (Jilek and Crites, 1982; Dunn *et al.*, 1983), inflammation (Measures, 1988; Molnár *et al*, 1993), granulomas (Hauck and May, 1977; Sindermann, 1990), ascites (Bauer *et al.*, 1977) and mesenteric and visceral adhesions (Sindermann, 1990). The pathogenic effect depends on the species and the size and the number of parasites (Fig. 12.3), and survival of the fish also depends on the site of infection.

Mortality

Mortality caused by nematodes was described by Bauer and Zmerzlaya (1972), who indicated that *R. acus* larvae caused heavy mortalities in bream (*Abramis brama*). According to Bauer *et al.* (1977), the heavily infected bream lost their balance and swam on their side, their body was covered by a thick layer of slime, there was local destruction of their sexual glands and bloody exudates accumulated in their abdominal cavity. Eiras and Reichenbach-Klinke (1982) also described heavy infection and deformation of the rainbow trout's intestine due to large parasitic nodules caused by *R. acus*. Moravec and Gut (1982) and Moravec *et al.* (1984) reported on the mortality of ornamental fishes due to massive infection with *Pseudocapillaria brevispicula* and *Capillaria pterophylli* (Fig. 12.5).

Schäperclaus (1992) also found that *Pseudocapillaria tomentosa* can severely damage tench (*Tinca tinca*). A devastating effect of nematode infection was observed by Molnár *et al.* (1991, 1993), who reported on massive eel mortality in Lake Balaton, Hungary, due to *A. crassus* (Figs 12.15 and 12.16) infection. In this lake an estimated 400 t of eels died in 1991, 1992 and 1995. Similar heavy mortalities caused by this nematode were observed in the Czech Republic by Barus (1995). *Goezia* spp. infecting the stomach of the fish seem to have a relatively high pathogenic effect. These worms bore their anterior ends deep into mucosa up to the muscularis layer. Gaines and Rogers (1972) observed tha

they form deep nodules in the stomach wall. These authors also reported mortalities in striped bass (*Morone saxatilis*) and tilapia in Florida. Mortalities were observed by Freitas and Lent (1946) in *Arapaima gigas* in Brazil caused by *Goezia spinulosa*.

Trichuroid nematodes are generally considered pathogenic. These nematodes damage epithelial cells by feeding and penetrating deeply into the intestinal mucosa. The necrotic changes they cause often lead to morbidity of the hosts. Dubinin (1952) in Russia reported on disease through gut inflammation of acipenserids (*A. ruthenus*, *Acipenser nudiventris*) caused by *Pseudocapillaria tuberculata*. Severe debilitating conditions also developed on some North American centrarchid fishes. *Capillaria catostomi* caused enteritis in the caeca and intestine (Hoffman, 1982). *Eustrongylides* spp. are also pathogenic. The pathogenicity of *Eustrongylides* spp. in sturgeons (*A. nudiventris*) was studied by Dubinin (1952) and Dogiel and Bykhovskiy (1939) in Russia, who considered *Eustrongylides* larvae to be highly pathogenic, causing heavy infections leading to complete destruction of gonads and to parasitic castration of infected fishes. It was also Dubinin (1952) who found that, in heavy infections with *H. bidentatum*, inflammatory processes were found in the intestinal walls of sterlets (*A. ruthenus*) and sometimes perforation of the swim bladder by migrating worms was recorded. Kall *et al.* (2004), who examined *P. obturans* infection of pike (*Esox lucius*), reported that the pikes infected by this large worm inhabiting gill arteries were less active, showed lethargy and died in aquaria shortly after transportation to the laboratory. Sprengel and Lüchtenberg (1991) experimentally proved that *A. crassus* infection in eels reduced swimming performance, and in another experiment Molnár (1993) found that heavily infected eels were more susceptible to decreased oxygen content in the water.

Intestinal tract

Most Nematoda species infect the intestinal tract. Some of them (e.g. *Hysterothylacium*, *Goezia*, *Cystidicola* spp.) prefer the anterior part of the alimentary tract (oesophagus, stomach, pyloric region), while others are in the intestine. The major damage caused by these worms is associated with their consumption of intestinal contents, thereby depriving the host of nutrients. A less important effect comes from direct mechanical blockage by the worms.

H. bidentatum (Fig. 12.2), a common parasite of the acipenserids, may cause heavy infections in sterlet. These large-sized nematodes can completely occlude the stomach and thus reduce digestion and block the passage of food. After the death of heavily infected fish, these nematodes frequently migrate out through the mouth or the gill slit.

In less intensive infections, nematodes may evoke pathological changes, mostly around their attachment sites. Damage to the mucosa or deeper tissues is usually caused by lips, the buccal capsule, teeth or spines.

Rhabdochona species, when in high numbers, can cause perforation of the intestinal wall at attachment points (Moravec, 1975). Similar damage occurs with *Camallanus*, *Procamallanus* and *Paracamallanus* species as they 'grab' the intestinal wall with their buccal capsules while feeding on blood. Usually there is a local inflammatory reaction at the attachment site. Thatcher (1991), as well as Sinha and Sinha (1988), suggested that nematodes could cause primary anaemia by feeding on blood. In intensive infections, especially in small fishes, these camallanids can reduce growth rates and also cause intestinal blockage. More severe changes were recorded in ornamental fishes. Several authors (Petter *et al.*, 1974; Stumpp, 1975; Campana-Rouget *et al.*, 1976; Schäperclaus, 1992) reported on complete destruction of the intestinal mucosa and death of the fish in the presence of large numbers of *Camallanus fotedari* or *Camallanus moraveci*. Heavy infection with *Camallanus cotti* caused a reduced sexual display rate in *Poecilia reticulata* (McMinn, 1990).

Cucullanus truttae has similar effects on rainbow trout. According to Dunn *et al.* (1983), there is loss of epithelium and mucosal hyperplasia, as well as haemorrhage and fibrosis in the laminar propria at the point of attachment. Growth rate, food consumption and swimming activity are reduced in infected fish. The spiruroid nematodes *Camallanus oxycephalus* and *Spinitectus carolini* of the green sunfish penetrate to the mucosal layer of the gut and cause damage to the columnar epithelium. At the site of penetration, ulcers developed in the mucosal and submucosal layers and there was growth of granulomatous tissue with extensive fibrosis (Meguid and Eure, 1996). Local changes in the intestine can also be provoked by seemingly less pathogenic nematodes.

In the case of *Echinocephalus daileyi*, where there is a special cephalic inflation and rows of hooks for attachment of the worm to the intestinal mucosa, Thatcher (1991) observed inflammation and formation of a fibrous capsule around the head bulb. Formation of capsules filled by tissue debris, oedematous fluid, fibrous exudate and leucocytes at the attachment point of the nematodes was also observed by Ko *et al.* (1975) with *Echinocephalus sinensis* in the ray *Aetabulus flagellum*.

Deardorff and Overstreet (1980) remarked that *Goezia pelagia* apparently feeds on both the host elements and partially digested food. The parasite forms a deep nodule in the intestinal wall, with the development of ulcers. A connective-tissue capsule surrounds the head part of the parasite, and primary exudates, inflammatory cells, red blood cells and necrotic tissue are in the nodules. *Aeromonas* bacteria could be cultured from some of the nodules. It is often larval penetration and migration that cause severe reactions. Thus, invasion of host tissue by marine nematode larvae has been described to cause pathological reactions. Haemorrhages, tissue compression and necrosis are often found in tissue with invading *P. decipiens* larvae (Ramakrishna *et al.*, 1993). The host reaction may be expressed in tissue proliferation, degeneration and inflammation.

Molnár (1994) found hundreds of *A. crassus* larvae in nodules in the intestinal wall; some of the larvae were alive while others were dead and calcified. Similar observations were reported by Janiszewska (1939) with *D. minutus* in flatfishes. Jilek and Crites (1982), who studied the pathogenicity of the habronematoid *S. carolini* in centrarchid fishes, described the third-stage larvae penetrating the intestinal wall, causing traumatic enteritis, the growth of epithelioid fibroblasts around worms and accumulation of granule cells, leucocytes and macrophages. An expanding fibrocytic layer formed a capsule around the larvae; the innermost layer became necrotic but encapsulated worms were able to develop into adults. *A. simplex* larvae have been seen to induce severe inflammatory reactions in the wall of the stomach of cod. Thus clusters of larvae gathered in local inflammatory foci in the stomach wall of the fish host (Berland, 1981). Arai (1969) found large ulcers caused by *Anisakis* larvae in the stomach wall of *Ophiodon elongatus*, and Williams and Richards (1968) observed prominent host reactions in *Raja radiata* against *Pseudanisakis rotunda*, especially the head, which was in the lamina submucosa granulation tissue.

Body cavity

The body cavity is the frequent location of *Philometra* and *Philonema*; however, little is known about their pathogenic effect. *Philonema* spp. are known to be responsible for multiple mesenteric and visceral adhesions in salmonid fishes (Nagasawa, 1985; Garnick and Margolis, 1990; Sindermann, 1990). Both *Philonema* and *Philometra* infection can cause atrophy or destruction of gonads, ascites and extension of the abdomen (Molnár, 1966a, 1967; Platzer and Adams, 1967; Williams, 1967; Hoffman, 1975; Moravec *et al.*, 2003). While studying *Eustrongylides* sp. larvae infecting mesenteries and inner organs of African fishes of the genera *Haplochromis*, *Bagrus* and *Clarias*, Paperna (1974) found that the most heavily infected fish were emaciated. In these fish an extensive lysis was observed around the worms, which penetrated the somatic muscles. Inner organs were infiltrated by lymphocytes and macrophages and inflammatory necrosis was observed

Eiras and Rego (1988) reported on similar changes in South American fishes (*Pygocentrus nattereri*), while Measures (1988) in North America found granulomatous inflammation and exudates containing erythrocytes and macrophages. However, Kennedy and Lie (1976) reported that heavy infections of encapsulated *Eustrongylides* did not cause weight loss or changes in the outward appearance and condition factor of fish. Experimental infections of rainbow trout with *A. simplex* third-stage larvae are known to cause a pronounced cellular reaction in the body cavity and in tissue penetrated, especially in the pyloric caeca (Santamarina *et al.*, 1994; Larsen *et al.*, 2002). Larvae of the genera *Hysterothylacium*, *Anisakis*, *Pseudoterranova* and *Contracaecum* and of *Spiroxis contortus* are frequently found on the serosa of the gut, where they are coiled and covered only by a thin serosa layer; in other cases they are encapsulated by a row of connective-tissue layers (Smith and Wootten, 1978).

Liver

Of the nematodes damaging the liver, *Schulmanela* (*Hepaticola*) *petruschewskii* is the best-known species. Kutzer and Otte (1966) studied the pathological effects of *H. petruschewskii* in salmonid, percid and cyprinid fishes. Macroscopic changes of the severely infected fish included greyish discoloration of the liver, with the appearance of nodules the size of a pinhead or larger, which was sometimes accompanied by hyperaemia, petechial haemorrhages and icterus. Histologically, in addition to the presence of helminths in numerous convoluted passages, haemorrhage and hyperaemia of the liver capillaries, aggravated by the appearance of fibrinous–serous exudate, were found. Leucocytic infiltration, epithelioid cell proliferation and even the appearance of giant cells could also be observed.

Devastating effects on host liver tissue were described by Petrushevski and Shulman (1961). Nematode larvae, probably *C. osculatum*, which normally reside in the liver of Baltic cod (*G. morhua*), were suggested to affect the size and function of the liver. Thus, heavy parasite burdens were seen in small livers and only a few nematode larvae were in large livers. Although tissue-penetrating worms may cause adverse reactions, further studies should be conducted to elucidate this question. In this context it should be recalled that liver size in gadoids is highly influenced by food composition and energy content (Buchmann and Børresen, 1988). Thus, feeding on low-energy diets such as crustaceans (serving as an intermediate host for *C. osculatum*) instead of high-energy feed (clupeids) may lead to high parasite burdens and, secondarily, low liver weight.

Of the nematodes causing heavy pathological changes of the liver in the larval stage, *R. acus* is the best-studied species. In the adult stage this parasite infects the gut of piscivorous fishes, primarily pike. In the latter fish, only local inflammations were registered in the gut. In intermediate host fishes, however, heavy infections and mass mortalities were often registered in rainbow trout, common bream, yellow perch and experimentally infected loach (*Barbatula barbatula*) (Moravec, 1970; Bauer *et al.*, 1977; Poole and Dick, 1984). Third- and fourth-stage larvae in their true intermediate hosts invaded various internal organs, destroyed blood vessels during larval migration and caused numerous nodules in the intestinal wall, liver, peritoneum and mesentery. Valtonen *et al.* (1994), who followed *R. acus* migration in roach (*Rutilus rutilus*), recorded a chronic granulomatous inflammatory reaction. They remarked that larvae occurred more often in pancreatic tissue than in the liver. These authors also remarked that the infection rate in polluted eutrophic lakes was much higher than in oligotrophic ones. Dezfuli *et al.* (2000), who studied the effect of larval raphidascariosis in the liver of *Phoxinus phoxinus*, reported that the inflammatory process was characterized by an increase of rodlet cells, besides granulocytes and epithelioid granulomata.

Authors who examined the effect of *Anisakis* larvae on host tissues (Mikailova *et al.*, 1964; Prusevich, 1964; Hauck and May, 1977; Smith, 1984) reported that *Anisakis* larvae invading tissues caused destruction of the liver parenchyma, ruptures of the wall of the blood vessels, small haemorrhages and thrombus formation. The larvae were covered with fibrin infiltrated by leucocytes. At a later stage, a connective-tissue capsule was formed around the larvae.

Swim bladder

The swim bladder of fishes is damaged mostly by *Cystidicola* and *Anguillicola* species. Van Banning and Haenen (1990) and Molnár *et al.* (1993) reported that the acute process of *A. crassus* larval migration was characterized by epithelial hyperplasia and hyperaemia of the swim bladder wall. In cases of chronic swim bladder inflammation, oedema and hyperplasia of tissues of the tunica propria, submucosa and serosa were observed, as well as granulomatoid infiltration by mononuclear cells and fibrinoid degeneration around larvae. Molnár (1994) remarked that inside the oedematous connective tissue of the swim bladder larvae migrate without causing an observable host reaction in the swim-bladder wall or the gas glands (Figs 12.17 and 12.18), but in more advanced cases granulation tissue containing mononuclear cells stops larvae (Fig. 12.19.) The granulation tissue, built up from epithelioid macrophages, forms a nodule around the larvae, which become surrounded by a capsule. Larvae die and, together with the necrotized epithelioid cells, give rise to amorphous tissue debris. Secondary bacterial infections may change nodules into pustules filled with degenerate cells, inflammatory cells and serum. Kamstra (1990), Van Banning and Haenen (1990) and Molnár *et al.* (1993) reported that adult *A. crassus* specimens filling the whole lumen of the swim bladder feed on blood, causing anaemia, epithelial hyperaemia and dilatation of the ductus pneumaticus. Due to the damage caused by larvae and adult worms, the wall of the swim bladder thickens and becomes fibrotic (Fig. 12.15). Worms in the lumen either leave the bladder and migrate to the gut through the ductus pneumaticus or they die. Dead worms and blood form a hard brown-black mass in the lumen of the swim bladder (Fig. 12.16), which will eventually contain facultative pathogenic bacteria.

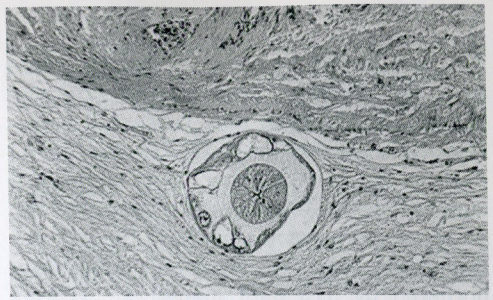

Fig. 12.17. Cross-section of a third-stage *A. crassus* larva in the oedematous tissue of the submucosa of the eel's swim bladder (H & E × 145).

Fig. 12.18. Fourth-stage larvae of *A. crassus* accumulating in the gas gland, before entering the lumen of the swim bladder (× 12).

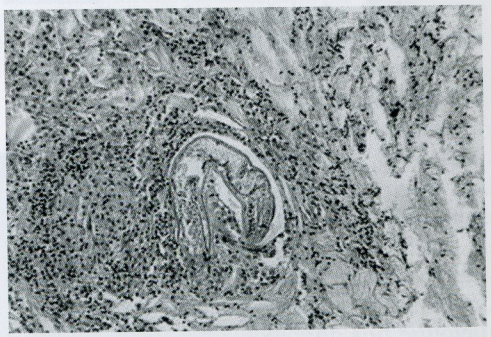

Fig. 12.19. Cross-section of a third-stage *A. crassus* larva inside the swim-bladder wall surrounded by granulation tissue and mononuclear cell infiltration (H & E × 85).

Skin

The best-known parasites of the skin come from the genera *Philometra* and *Philometroides*. By the time they reach full maturity, the large females of *Philometra rischta* destroy the subepithelial layer of the gill cover of bleak (*Alburnus alburnus*), and the worms are separated from both the gill and the aquatic environment by an epithelium bordered by basement membrane. Worms cause ulceration of the gill cover, which becomes completely disintegrated in some cases (Molnár, 1966a; K. Molnár, unpublished). Similar injuries may arise after colonization of the skin of sucker (*Catostomus commersonii*) by *P. nodulosa* (Dailey, 1966; Hoffman, 1975, 1999). The very tiny *S. cyprini* is not pathogenic, while the large females (up to 20 cm in length) of *Philometroides cyprini* (Fig. 12.4), a parasite of the Amur wild carp, can cause swellings of the scale sacs, accumulation of serous infiltration and haemorrhages under the scales. After the expulsion of worms, the areas without scales and with ulcers may have secondary microbial infections (Vasilkov, 1967, 1975; Sekretaryuk, 1983). Similar changes in the fins are caused by *P. sanguinea* infection in *Carassius* species (Fig. 12.20) and by *Philometra huronensis* infection in sucker. These changes lead to fraying of the fin rays, which may break and come off in fragments (Molnár, 1966a; Uhazy, 1978). In sturgeon fishes, especially in the sterlet, *C. acipenseris* forms large nodules in the connective tissue on the ventral surface of the skin (Fig. 12.21), causing capsule formation and oedema (Bauer *et al.*, 1977). There is usually a female and a male worm in each nodule. Rockfish infected with adult trichuroid nematodes in the skin have inflammatory reactions with intra-epithelial deposition of eggs (Conboy and Speare, 2002).

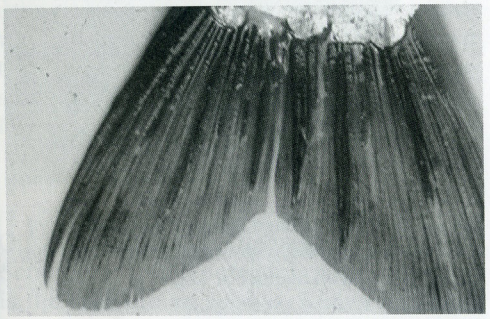

Fig. 12.20. Females of *Philometroides sanguinea* in the blood vessels of the caudal fin of a gibel carp (*Carassius gibelio*) (× 0.85).

Gills

Nematode infection of the gills is relatively rare. The best-known nematode is *P. obturans*, a parasite of the gill arteries of the European pike. This large-sized nematode, measuring 20 cm in length, is known to obstruct blood circulation, to feed on blood and to perforate gill arteries when releasing larvae. Moravec and Dyková (1978) and Kall *et al.* (2004) found parietal trombi in the bulbus arteriosus and endothelial hypertrophy in the ventral aorta. The worms clearly obstructed the arteries; the elastic wall of the vessel was stretched around the parasite and appeared thinner. In cases when the female worm was damaged and only the gonads filled the arteries, the arterial wall became irregular, showing signs of hypertrophy and hyperplasia. In contrast to this large Nematoda species, Ribu and Lester (2004) found tiny histozoic nematodes in gill filaments and described them as *Moravecia australiensis*.

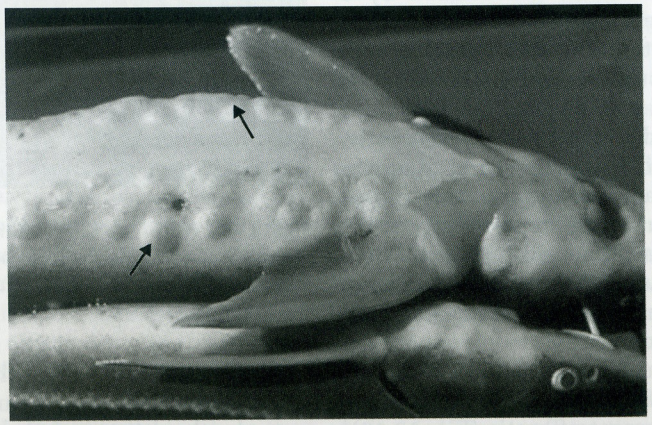

Fig. 12.21. Parasitic nodules (arrows) in the skin caused by *Cystoopsis acipenseris* in the abdominal side of sterlet (*Acipenser ruthenus*) (× 0.4). Photo by Ferenc Baska.

Eyes

The eyes of fishes may frequently have worms. For example, Parukhin (1975) found the females of *Philometra oveni* in the orbits of a percid fish (*Serranelus hepatus*) and Moravec and Dyková (1978) found larval stages of *P. obturans* in the vitreous humour.

Zoonotic aspects of fish nematodes

The presence of nematode larvae in fish may present a risk for humans. Third-stage larvae of *Anisakis* spp., *Pseudoterranova* spp., *Phocascaris* spp. and *Contracaecum* spp. occur in fish musculature and their adult stage is in homeothermic animals such as whales and seals. Thus, the stimuli for further development of the larva to the final moult are high temperature, acidic pH and pepsinogen, which occur in the stomach of homeothermic animals. Following ingestion by humans of live larvae in fish products, the larvae get activated and penetrate the gastric or intestinal mucosa, eliciting abdominal symptoms. Extra-intestinal migration of worms also occurs. This is anisakiasis (anisakiosis) or pseudoterranoviasis (pseudoterranovosis) (Smith and Wootten, 1978; Möller and Anders, 1986; McClelland, 2002).

Freezing (–20°C for 24 h), heating above 60°C or salting (250 g NaCl/l) will kill *Anisakis* larvae. Likewise, storage of *Pseudoterranova* larvae in fish meat at –30°C for 15 h or at –20°C for 7 days is lethal for the worms (McClelland, 2002). Even dead worms (killed by freezing, heating or salting) still contain immunogenic molecules, which can trigger severe allergic reactions following ingestion of fish products. In fact, the disease provoked by the marine larvae is to a high degree associated with the host reaction, such as immunoglobulin E (IgE) production, mast-cell degranulation, eosinophilia, oedema and urticaria. It has been speculated that nematodes living in the adult stage in fish, such as *Hysterothylacium*, could elicit disease in humans following ingestion of live larvae (Norris and Overstreet, 1976). It has been reported that adult nematodes of *Philometra* from fish tissue can infect humans through open wounds in skin and elicit disease (Deardorff *et al.*, 1986). Considering the high prevalence of philometrids in fishes (Moravec *et al.*, 2003), this suggestion should be further studied.

Spoilage of fish products by marine nematode larvae

Nematode larvae in fish fillets may lead to rejection by consumers due to the unattractive appearance of the product. Therefore considerable efforts are being made to detect and remove larval nematodes from fish products (Möller and Anders, 1986). It has been estimated that up to half of the production costs in certain processing plants consist in the detection and removal of *Pseudoterranova* from cod fillets; the problem is worldwide due to the extensive distribution of these anisakid larvae (McClelland, 2002). These concerns are mainly with wild fishes because several studies have shown that aquacultured salmon are free from infection, since in this case fish conduct their entire life in captivity and are fed with non-infected feed (Lunestad, 2003).

In Vitro Culture of the Parasite

Studies on the *in vitro* culture of marine nematodes have concentrated on marine anisakids. Thus, incubation of third-stage larvae of *A. simplex* (Carrajal *et al.*, 1981), *P. decipiens* (Likely and Burt, 1989) and *C. osculatum* (Likely and Burt, 1992) in appropriate physiological media containing serum, haemin and amino acids resulted in moulting and in some cases in development of adult egg-producing worms. Similar successful results were obtained with *in vitro* culture of *H. aduncum* from third-stage larvae to adult worms by Iglesias *et al.* (2002). The process was developed by Adroher *et al.* (2004), who managed to follow the development process of *H. aduncum in vitro* from the third-stage larvae to the hatching of the new generation third-stage larvae. Further studies could also focus on the culture of all stages for a repeatable, complete *in vitro* life cycle.

Identification and Diagnosis of Infection

General methods for identifying nematodes are described in textbooks by Bykhovskaya-Pavlovskaya (1969), Fernando *et al.* (1972), Bauer *et al.* (1977), Bauer (1987), Sindermann (1990) and Moravec (1994).

Methods for diagnosis of nematodes much depend on the size and location of the parasites. In live fish, only large parasites on or close to the surface of the body are recognized. Red-coloured *Philometra* species in the opercula and the fins or parasite nodules in the skin around *C. acipenseris* can easily be observed. Large nematodes inhabiting the gut and inner organs are easily detected on dissection of freshly killed, frozen or formalin-fixed animals; in the case of small nematodes, a dissecting microscope is necessary. Infection with histozoic species, such as the small skrjabillanid nematodes, can be diagnosed on live material under a microscope. Scrapings of intestinal serosa can be examined under a dissecting microscope for the delicate capillariid nematodes and larval stages. Larval nematodes in the internal organs are usually found in squash preparations between two glass plates. Physiological 0.6% fish saline is necessary to keep nematodes alive. Before fixing, nematodes are rinsed in saline. For fixation, a hot mixture of 70% ethanol and glycerine (9 : 1 part) or a hot mixture of saline and 40% formalin can be used. For diagnosis of *Anguillicola* infection in the lumen of the swim bladder, Beregi *et al.* (1998) suggested X-ray (Fig. 12.22) and Székely *et al.* (2004) used computer tomographic methods.

Male genital organs can be studied by placing the worms in glycerine or lactophenol under a cover slip for clearing. For staining permanent mounts, carmine staining

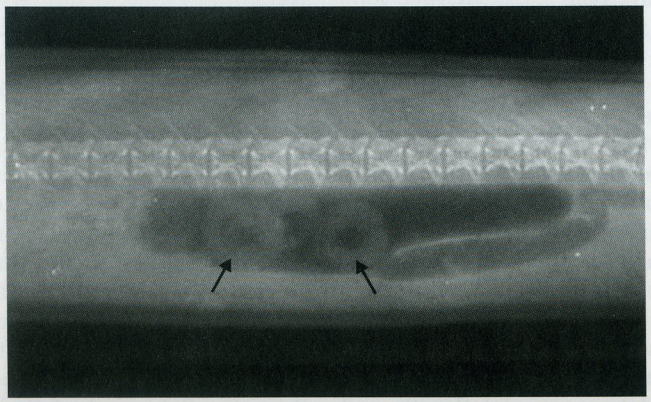

Fig. 12.22. X-ray as a tool for diagnosis of *A. crassus* infection of eel swim bladder. Note the large convoluted worms (arrows) in the swim bladder and the ductus pneumaticus (× 1).

or Thatcher's method (Petter and Thatcher, 1988) is suggested. Oral papillae can be studied by *en face* preparations (e.g. as suggested by Anderson, 1958).

Identification is based on morphological characters. Thus, relative proportion of length and width is used. An important feature is the overall shape of the worm, specifically the presence of papillae, alae, boring tooth, striations, oral opening, excretory pore opening location, caeca and appendages on oesophagus, ventriculus and intestine. As an example, it can be mentioned that the identification of a number of species (*P. decipiens*, *Pseudoterranova krabbei*, *Pseudoterranova bulbosa* and *Pseudoterranova azarasi*), comprising a species complex previously referred to as *P. decipiens* (Paggi *et al.*, 2000), has traditionally been based on such structures. Morphologically, the worm is characterized by an anteriorly directed intestinal caecum, no ventricular caecum and an excretory pore opening at the nerve ring. Most ascaridoids are clearly differentiated from *Anisakis* spp. *Anisakis* larvae are easily identified by the lack of caeca on the intestine and ventriculus. Likewise a characteristic tail spine is found. The excretory pore is located anterior to the nerve ring (Smith and Wootten, 1978). The differential diagnosis of *C. osculatum* is also feasible using morphology. Both ventricular and intestinal caeca are found and the excretory pore opens anterior to the nerve ring. Species of *Porrocaecum* and *Phocascaris* are difficult to distinguish morphologically, but the former genus is presumed to be in birds while the latter genus is in seals (Paggi *et al.*, 2000). Some similarity exists between *Contracaecum* spp. and *Hysterothylacium* spp. larvae. However, several differences are found, among which the location of the opening of the excretory pore is important. The third-stage larva of *Hysterothylacium* has no lips and carries characteristic caeca on both the intestine and ventriculus, but the excretory pore opens below the nerve ring (Fagerholm, 1982). Likewise, morphological differences are obviously used to diffentiate adult nematodes in fish. The identification of cucullanids is based on the shape and length of the worms, the location of the vulva and the shape of the spicule (Berland, 1970).

Molecular tools

The recent development of molecular techniques has provided alternative and, in some cases, more accurate diagnostic tools. Thus, PCR-based methods include amplification of genes encoding ribosomal RNA. Direct alignment and comparison of DNA sequences may reveal differences between species. By using the PCR-restriction fragment length polymorphism (RFLP) method, various closely related nematodes can be differentiated on their banding pattern in an agarose gel (Kijewska *et al.*, 2002; Szostakowska *et al.*, 2002). Further, mitochondrial DNA sequences have been found to be valuable for differentiation between sibling species within *Contracaecum ogmorhini* (Mattiucci *et al.*, 2003). In addition, electrophoresis of enzymes from various nematodes and subsequent comparison of gel migration distances allow separation of such species. These techniques were also used for confirmation of mitochondrial DNA results from *C. ogmorhini* studies (Mattiucci *et al.*, 2003). These have been found to be feasible for differentiation between the genera *Anisakis, Contracaecum* and *Pseudoterranova* (Mattiucci *et al.*, 1998). *P. decipiens* was formerly considered to be a single, well-defined species, but recent allozyme work has shown that this entity comprises a species complex like *P. decipiens* (*sensu stricto*), *P. krabbei, P. bulbosa* and *P. azarasi* (Paggi *et al.*, 2000). Similar techniques were used by Mattiucci *et al.* (2002) for differentiation between *Anisakis typica* and other species within the genus.

Prevention and Control

Parasitic nematodes primarily affect fish in natural waters. Only a smaller proportion of them cause problems in fish ponds and aquaria.

Lo (1988) summarized the methods for treating the valuable discus fish (*Symphysodon*) in an aquarium. Anthelmintics, such as garlic oil, piperazine, trichlorphon and levamisole, have varying effectiveness when administered in food. He suggested trichlorphon in a bath (concentration of 4 ppm or 7 ppm), but several side effects were recorded. Pena *et al.* (1988) found that fresh minced garlic (200 mg/l water) and its hexane extract were 100% effective in treating *Capillaria* in carp, while ammonium potassium tartrate (1.5 mg/l, twice daily) was 86% effective.

Avdosev (1978) suggested that *Philometroides lusiana* could be controlled during the larval shedding period by killing the intermediate host cyclops in the pond with 0.325 g/m^3 trichlorphon administered three times at 10-day intervals. Vasilkov *et al.* (1974) found that ditrazine citrate, administered in an oral dose of 0.4 g/kg of fish, was effective against *P. cyprini* (*P. lusiana*) developing in common carp, and the same drug was effective even in a dose of 0.3 g/kg when inoculated into the abdominal cavity.

The effects were examined of a few anthelmintics against *A. crassus* infection of the eels. Taraschewski *et al.* (1988) tested five drugs but only levamisole-HCl proved to be effective. Hartmann (1989) found that levamisole in bath treatment was effective in 2 and 5 mg/l doses.

Controlled studies by Tojo *et al.* (1994) showed that experimental infections of rainbow trout with *A. simplex* third-stage larvae were only weakly influenced 6 days post-treatment by anthelmintics such as mebendazole, flubendazole, parbendazole, triclabendazole, piperazine, netobimin, trichlorphon and nitroscanate. However, previous *in vitro* studies have shown effects of mebendazole, flubendazole, triclabendazole, nitroscanate and various types of Chinese herbal medicine on *Anisakis* spp. larvae (Tojo *et al.*, 1994). Other *in vitro* studies showed clear effects of ivermectin on larvae and adults of *P. decipiens* (Manley and Embil, 1989). Therefore, it is likely that some anthelmintics used to treat nematode infections in humans and animals may be effective against nematodes of marine animals if dosage and exposure time are appropriate. Benzimidazoles, pyrantel, morantel, avermectins and levamisole are all drugs with high nematicidal potential.

Prophylaxis

Bauer and Hoffman (1976) as well as Molnár (1987) emphasized the importance of the control of intercontinental transfer of fishes. These authors, as well as Bauer *et al.* (1981) and Schäperclaus (1992), recommended that all wild fish should be examined for internal and external parasites before stocking. Parasites spread by fish-eating birds can be the most effectively controlled if the definitive host birds are kept away from ponds or the water supply containing intermediate hosts. Bauer *et al.* (1981) suggested that, at the time of larval release of *P. cyprini*, brood fishes should be isolated from susceptible young fish.

Heat inactivation (60°C) or freezing (–20°C for 24 h) will kill infective third-stage larvae of *Anisakis* spp. (Smith and Wootten, 1978). Likewise, *Pseudoterranova* larvae are killed in fish products by storage at –30°C for a minimum of 15 h or at –20°C for at least 7 days (McClelland, 2002). Immunoprophylaxis in fish against nematode infections has not been achieved, although it is known from veterinary immunoparasitology that immune reactions in farm animals can eliminate infections. We suggest that it is time to initiate studies on developing vaccines against some of the more important pathogenic nematodes in fishes.

Summary and Conclusions

Knowledge of fish nematodes varies greatly. The nematode fauna of fish in Europe and the northern parts of Asia and America is relatively well studied. Little is known, however, about fish nematodes of South Asia, Africa, Australia and Latin America. All nematodes in marine fishes with zoonotic relevance have been studied and, of these, large-sized ascaridoid and philometrid nematodes are the best known.

Small-sized nematodes, such as the skrjabillanids, are known mostly from Europe, although recent studies on fishes of Central America show that their distribution may also be high in other continents. In most cases, only the intestinal tract is examined and histozoic nematodes infecting inner organs, serous membranes and muscles may not be found by inexperienced examiners.

There is an urgent need for the introduction of molecular methods in the research of fish nematodes. This method can be useful in synonymizing or separating morphologically similar species and finding latent infections with small histozoic nematodes.

Pathogenicity of fish parasites is mostly based on field observations, except studies done on *A. crassus* infections. *R. acus* and *A. simplex* also seem to be experimental tools for studying pathogenic effect of nematodes.

There is no effective drug against fish nematodes. Although nematodes cause relatively few problems in propagated fishes in fish farms or cage cultures, they are relatively important in aquarium fishes.

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